

Pre-planting inoculation for early establishment of *Dicyphus bolivari* and *D. errans* on tomatoes

Filipe Madeira · Eva Edo · Samuel Sossai · Joan Callizo · Ramon Albajes

Received: 15 June 2018 / Accepted: 29 October 2018
© The Author(s) 2018

Abstract Slow establishment and risks of plant damage are major constraints for the use of the predatory mirid bugs that are commercially available for release in greenhouse crops. Therefore, a new interest has turned towards two predatory *Dicyphus* species (Heteroptera: Miridae) that have been investigated in the past but have not been used commercially in augmentative biological control. We assessed their development duration and survival at temperature and day length of summer and winter conditions and assessed the feasibility of pre-planting predator establishment on plant seedlings under those conditions. *Dicyphus bolivari* (Lindberg) and *D. errans* (Wolff) nymphs reached the adult stage in 47.4 (winter conditions) and 18.6 days (summer conditions) and 46.0 (winter) and 16.3 days (summer) respectively. They showed more than 90% survival when provided with food (*Ephestia* eggs). Provision of food allowed females to lay more eggs and to distribute them more

evenly among tomato seedlings. No injuries or other negative effects on tomato seedling development were observed. Nymph development time of the two mirid species studied were slightly higher than other commercial mirid predators. The results show great potential of these predatory bugs, which are now being tested under field conditions.

Keywords Hemiptera · Miridae · Biological control · Mirid bugs · Whitefly

Introduction

Most integrated pest management programmes in the greenhouse industry in Europe are based on biological control (van Lenteren 2012). This strategy was first practised in the Netherlands and in England in the 1960s (van Lenteren and Woets 1988), with the release of the whitefly parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae), the spider mite predator *Phytoseiulus persimilis* (Athias-Henriot) (Acari: Phytoseiidae) and, more occasionally, leaf miner parasitoids and aphid natural enemies (van Lenteren and Woets 1988). After some pioneering biological control applications in the Mediterranean basin in the 1980s (Albajes et al. 1996; Casadevall et al. 1979; Onillon 1990), the method spread very fast in southern Spain from the mid 2000s. The agricultural area covered by repeated releases of natural enemies has

Handling Editor: Ralf Ehlers.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10526-018-09911-3>) contains supplementary material, which is available to authorized users.

F. Madeira (✉) · E. Edo · S. Sossai ·
J. Callizo · R. Albajes
Universitat de Lleida, Agrotecnio Centre, Rovira Roure
191, 25198 Lleida, Spain
e-mail: fmadeira@pvcf.udl.cat

risen from a few hectares in 2005 to more than 70% of the protected vegetable gardening area around Murcia and Almeria (Bueno and van Lenteren 2010; van Lenteren et al. 2019). As predicted by Albajes and Alomar (1999), biological control in the Mediterranean was soon based on generalist natural enemies such as mirid bugs and flower bugs (Heteroptera: Anthocoridae) (Riudavets and Castañé 1994). Predatory bugs (Hemiptera: Miridae) used in greenhouses are omnivorous, that is, they may feed on plants and prey with different facultative behaviours (Coll and Guershon 2002), a phenomenon quite common among predatory insects (Albajes and Alomar 2004). Two mirid bugs, *Macrolophus pygmaeus* (Rambur) and *Nesidiocoris tenuis* (Reuter) (Hemiptera: Miridae), are being commercially used in Europe, particularly in Mediterranean regions. *Macrolophus pygmaeus* (formerly known and reported as *M. caliginosus* (Wagner), a species that should now be referred to as *M. melanotoma* (Costa) (Sanchez and Cassis 2018)), was initially the species predominantly released in Europe. However, since the introduction and spread of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in all Mediterranean tomato greenhouses, *N. tenuis* has been most commonly used because of its acceptable action against this and other greenhouse pests (Urbaneja et al. 2012). Both predatory species have some characteristics that limit their suitability for biological control. *Nesidiocoris tenuis* can injure the crop plant under certain conditions (Castañé et al. 2011), and both predators are slow to establish in the crop: they normally require five to eight weeks after release to reach a sufficient population density to keep the pest under control (Calvo et al. 2012a; Trottin-Caudal et al. 2012). To enhance predator establishment, provision of alternative prey like *Ephestia kuehniella* (Zeller) eggs has been practiced with good results (Messelink et al. 2014). Although several banker plants have been tested for a faster establishment of mirid bugs in protected crops, an alternative cheaper method has been achieved by releasing the predatory bugs in the nursery and allowing it to lay eggs on the seedlings, so that when the young plants are transplanted into the greenhouse a certain number of mirid bug nymphs are already installed (Calvo et al. 2012a; Lenfant et al. 2000; Perdakis et al. 2015). However, an excess of predatory bugs released in the nursery may cause plant damages later after transplant in the greenhouse as recorded for

N. tenuis when it is released at rates above 0.5 individuals per seedling (Calvo et al. 2012b).

In order to solve these drawbacks, interest has turned towards two species of predatory mirids that have been investigated in the past without being used commercially, *Dicyphus tamaninii* (Wagner) (Heteroptera: Miridae) (e.g., Albajes et al. 1996) and *D. errans* (Wolff) (e.g., Tavella et al. 1997). *Dicyphus tamaninii* was first investigated in the early 1980s for use as a predator in Mediterranean greenhouses (Gabarra et al. 1988). Since then, several studies have elucidated many of its biological characteristics and its potential as biological control agent, with promising results. *Dicyphus tamaninii* is an omnivorous predator that may feed on crop plants, so risks of crop damage are a potential limitation for its use in commercial applications (Alomar and Albajes 1996; Barnadas et al. 1998). In a recent paper based on a study around the Mediterranean basin, Sanchez and Cassis (2018) revised the taxonomy of some of the species on which we had carried out our previous work and identified them as *D. bolivari* (Lindberg) [= *D. maroccanus* (Wagner)], as we had initially called this species in Casadevall et al. (1979), although we referred to it as *D. tamaninii* in later works. Therefore, we will refer to *D. tamaninii* as *D. bolivari*. The main biological features and predatory capacity of *Dicyphus errans* have also been studied (e.g., Ingegno et al. 2013), but less work has been devoted to analysing its usefulness for biological control in greenhouses (Ingegno et al. 2017). Since the two *Dicyphus* species are well established around the Mediterranean Basin, they can be used in conservation biological control if early entrance and establishment into greenhouses are favoured. On the other hand, the two species have different distributions in the Mediterranean areas: whereas *D. errans* is more frequently found in northern zones, *D. bolivari* has a more southern distribution. In this work, we aim, first, to assess whether these predators can be used in both Mediterranean summer and winter conditions and, second, to determine whether the release of mirid adults in seedlings to enhance early establishment results in egg laying without injuring the young plants.

Materials and methods

Plants and insects

Tomato plants (*Solanum lycopersicum* L., cv. Roma VF) were grown in a climate-controlled room (25 ± 2 °C, 16:8 h L:D photoperiod). Seeds were sown in peat substrate (pH = 6.0; NPK fertilizer content of 1.3 g l^{-1}) (Klasmann-Deilmann®, Geeste, Germany) in pots (ø 5.5 cm and 10 cm high) with no pesticide or fertilization applications, and were watered as needed. The predatory mirids *D. bolivari* and *D. errans* were reared under controlled conditions (25 ± 2 °C, 16:8 h L:D photoperiod) at the laboratory of Entomology at the University of Lleida – Agrotecnio Centre (Catalonia, Spain). Laboratory colonies were established from individuals provided by the Laboratory of Entomology of IRTA-Cabrils (Catalonia, Spain). Original rearing was made on tobacco plants, with *Ephestia* eggs as food and under standard conditions as mentioned above. The mirid colonies were reared in our laboratory in 2 l glass jars (30×15 cm) ventilated through a circular window of 10 cm in diameter and covered with muslin, fed *ad libitum* with frozen eggs of *E. kuehniella* (Lepidoptera: Pyralidae) (BIOCARE GmbH, Einbeck, Germany) and provided with water for drinking and freshly cut tomato stems and or recently harvested French green bean pods (*Phaseolus vulgaris* L.) as egg-laying substrates. Twice a week, the mirid colonies were provided with food and water and egg-laying substrates were replaced. Substrates with mirid eggs were placed into new rearing jars. Adults and nymphs were reared under the same conditions.

Nymphal development time

Nymph development time for the two mirid species (*D. bolivari* and *D. errans*) was measured under two conditions (1) 25 °C, 16:8 h L:D, and 55–70% RH and (2) 16 °C, 9.5 h L; 11 °C, 14.5 h D, and 65–75% RH. These conditions correspond to the temperatures and humidities which are common in Mediterranean areas during the summer and winter season, respectively. Daily, egg-laying substrates from mirid colonies were checked to monitor egg hatching. Newly hatched nymphs (< 24 h old) were individually put into transparent ventilated cylindrical containers (5.5 cm ø, 3 cm height). Nymphs were supplied with a tomato

leaf with (1) *ad libitum* *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) nymphs or (2) *ad libitum* *E. kuehniella* eggs or (3) a mixed diet with the two kinds of food. Survival and development of nymphs were checked daily until the adult stage and food was renewed every day. A minimum of 20 replicates for each species - type of diet - temperature were observed.

Inoculation of tomato seedlings with *Dicyphus* spp

Six potted tomato seedlings with three fully deployed leaves were placed in tents ($60 \times 60 \times 60$ cm, Insect Rearing BugDorm®-2120F, from BugDorm Store). Three treatments were compared for each of the two *Dicyphus* species: (1) tents with only plants but no mirid bugs in order to evaluate the development and condition of plants without any contact with mirid bugs, (2) with only plants and mirid bugs; without food provision, called the diet⁻ treatment; and (3) tents with plants and mirid bugs provided with food (two paper strips of 7.5×1.5 cm with *E. kuehniella* eggs glued to them and placed on the top of plant leaves), called the diet⁺ treatment. Two rates of mirid bugs were tested: (1) one individual per plant, i.e., three males and three females per tent containing six plants, and (2) two individuals per plant, i.e., six males and six females per tent containing six plants. Released mirid bugs were between six and eight days old which is the age when females start to lay fertile eggs (author's observation). The adult mirids were removed from the tents and the number of survived individuals were counted after five days. During the five days that the mirids were in the tent, injuries to the plants caused by them were recorded by visual observation. The vigor of plants was also measured by the number of compound leaves, the length of leaves and the number of leaflets per leaf. Measurements and counts were conducted at three times: before the release of mirid bugs, as well as one and two weeks after the removal of mirid bugs. Five days after the removal of the adult mirid bugs from the tents, the number of mirid bug nymphs on the plants was periodically recorded and nymphs were removed from the plant. After three consecutive days with no new nymph records, the experiment ended. Each tent was considered as a replication and there were six replications per experimental condition and *Dicyphus* species. Trials were

conducted in climatic chambers at standard conditions of 25 ± 1.5 °C, 16:8 L:D, and 55–70% RH

Data analysis

Statistical analyses were performed using R software version 2.15.0 (R Development Core Team 2012). The response variables, development time of nymphs (number of days from 1st instar to emergence of adult mirid bug), the evenness of eggs laid among plants, the coefficient of variation (distribution of nymphs per plant) and the length of leaves (cm) were analysed using generalized linear models (GLM) based on a Gaussian distribution with an identity link function. In the case of the response variable development time, for each condition, the explanatory variables used in the model were ‘species’ (Db vs. De) and ‘diet’ (*B. tabaci* vs. *E. kuehniella* eggs vs. mixed diets) as factor and the their interactions. In the case of the response variable coefficient of variation, the explanatory variables used in the model were ‘number of females released’ (3 vs. 6), ‘diet’ (diet[−] vs. diet⁺) and ‘species’ (Db vs. De) as factors and the their two-way interactions. For length of leaves, the explanatory variables used in the model were ‘number of females released’ (3 vs. 6), ‘treatment’ (diet[−] vs. diet⁺ vs. control) and ‘species’ (Db vs. De) as factors and their two-way interactions. Prior to these analysis, the data were tested for normality (Shapiro–Wilk test) and homogeneity of variances (Bartlett test), and when necessary data were transformed to $y = \log(x + 1)$. The response variables ‘offspring’ (number of nymphs) and ‘plant Leaves’ (number of leaves) were analysed using GLMs based on a Poisson distribution with a log link function. In the case of the response variable offspring, the explanatory variables used in the model were ‘number of females released’ (3 vs. 6), ‘diet’ (diet[−] vs. diet⁺) and ‘species’ (Db vs. De) as factors. The diet \times species interaction and the number of females released was also used as a co-variable. In the case of the number of leaves per leaflet, the explanatory variables used in the model were ‘number of females released’ (3 vs. 6), ‘treatment’ (diet[−] vs. diet⁺ vs. control) and ‘species’ (Db vs. De) as factors and their two-way interactions. The response variable ‘Survival’ (proportion of females survival in the tent after five days) was analysed using GLM based on a Binomial distribution with a logit link function. The explanatory variables used in the model were ‘number

of females released’ (3 vs. 6), ‘diet’ (diet[−] vs. diet⁺) and ‘species’ (Db vs. De) as factors and their two-way interactions. Models were simplified with a stepwise model selection based on the Akaike information criterion using the ‘stepAIC’ function of the MASS package in the R software (Venables and Ripley 2002). When significant differences were found, post-hoc comparisons of mean values were performed using the Tukey HSD method (agricolae package). The survival of nymphs was compared between diet regimes, climate conditions and species using a two-tailed Fisher exact test with the R software.

Results

Nymphal development time

Dicyphus bolivari and *D. errans* were able to reach the adult stage with high survival rates of nymphs, reaching values between 90 and 95% in both winter and summer conditions when fed with *B. tabaci* nymphs, *E. kuehniella* eggs or a mixed diet of both *B. tabaci* and *E. kuehniella* (Table 1). Diet, temperature, RH, and photoperiod did not affect survival of *Dicyphus* nymphs (Fisher exact test, $P > 0.05$). The development time of nymphs of predatory mirid bugs was significantly influenced by climatic conditions and type of food. In colder conditions, the development time of nymphs ranged from 44 to 49 days, showing significant differences between species and diet. *Dicyphus errans* nymphs developed faster than those of *D. bolivari* ($F_{1,114} = 7.57$, $P < 0.01$) and the two mirids reached the adult stage earlier ($F_{2,112} = 38.43$, $P < 0.001$) when fed on a mixed diet (Table 1). Similar results were obtained at warmer conditions, the development time of nymphs was significant different between species and diet. Development time of nymphs ranged from 16 to 19 days, *Dicyphus errans* nymphs developed faster than those of *D. bolivari* ($F_{1,132} = 72.17$, $P < 0.001$). The influence of the diet on development time of nymphs ($F_{2,130} = 30.24$, $P < 0.001$), in which individuals fed on a mixed diet reaching the adult stage first, followed by individuals fed on *E. kuehniella* eggs and individuals fed on *B. tabaci* nymphs (Table 1).

Table 1 Nymphal developmental time (days, mean \pm SE) of *D. bolivari* and *D. errans* under 16 °C, 9.5 h L; 11 °C, 14.5 h D, 65–75% of RH conditions (winter/autumn) and under 25 °C, 16:8 h L:D, and 55–70% of RH conditions (spring/summer) when fed on three types of diet: *B. tabaci* nymphs, *E. kuehniella* eggs, or a mixed diet with the two types of food

	Autumn/winter conditions N1-Adult (days)	Spring/summer conditions N1-Adult (days)
Species		
<i>D. bolivari</i>	47.41 \pm 0.50a	18.64 \pm 0.23a
<i>D. errans</i>	45.98 \pm 0.39b	16.28 \pm 0.24b
Diet		
<i>B. tabaci</i> nymphs	48.58 \pm 0.60A	18.95 \pm 0.34A
<i>E. kuehniella</i> eggs	48.03 \pm 0.42A	17.47 \pm 0.33B
<i>B. tabaci</i> + <i>E. kuehniella</i>	43.85 \pm 0.33B	16.04 \pm 0.22C

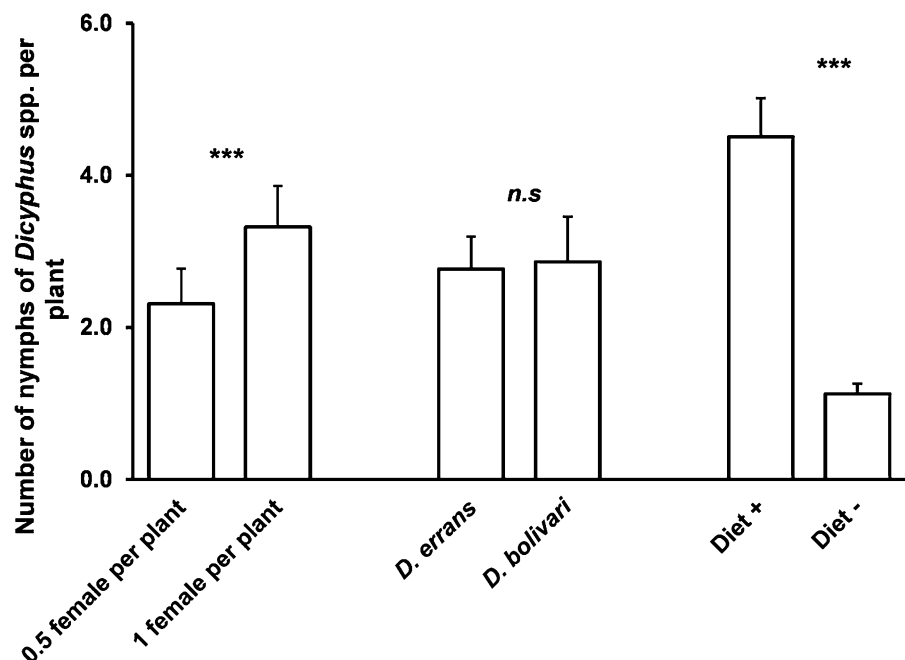
Means followed by different letters are significantly different. Means followed by the same lower or upper case letters within each weather condition are not significantly different between species or diets, respectively (Tukey HSD test, $P < 0.05$). The double Species \times diet interaction was not significant under any of the two conditions. A minimum of 20 replicates for each species, type of diet, and climate condition were used

Pre-planting inoculation of tomato seedlings with predators

The two *Dicyphus* species were able to lay eggs onto tomato seedlings when kept on them for five days. There were no significant differences in offspring between mirid bug species (Fig. 1). The number of females released and the presence or absence of *E. kuehniella* eggs significantly affected the number of

Dicyphus offspring (Fig. 1). When the number of adults released was increased twofold, the number of produced nymphs increased significantly (14 vs. 20 nymphs per tent, i.e. per six tomato seedlings, respectively). Also, the number of nymphs was four times higher when predator adults were provided with food (*E. kuehniella* eggs) than when no food was provided (Fig. 1). After five days, we recaptured more than 80% of the adults released in the tent. We

Fig. 1 Number of 1st instar nymphs (offspring, mean \pm SE) per tomato seedling when 0.5 and 1 females of *D. errans* and *D. bolivari* were initially released in tents with six tomato seedlings and fed with *E. kuehniella* eggs (Diet⁺) or not (Diet⁻) at 25 °C, 16:8 h L:D, and 55–70% of RH. Females were allowed to lay eggs on tomato seedlings for five days. Asterisks (***) indicate significant differences ($P < 0.001$) and n.s indicates not significant differences ($P > 0.05$)



observed no significant differences in the survival of adults released in relation to the number of released females ($\chi^2 = 0.18$, $df = 1$, $P = 0.67$) or provision of diet ($\chi^2 = 0.15$, $df = 1$, $P = 0.69$), or between species ($\chi^2 = 2.51$, $df = 1$, $P = 0.11$).

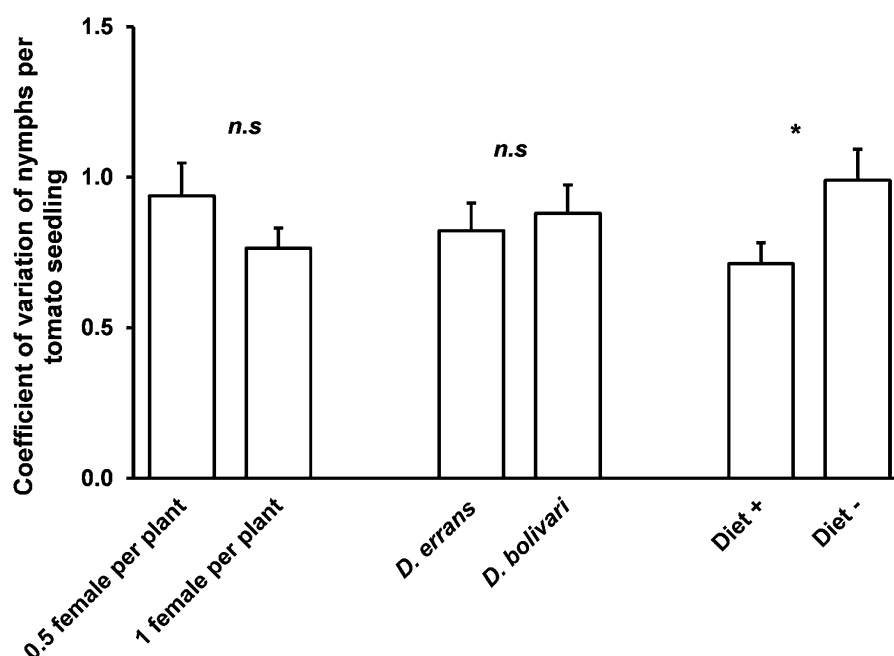
Distribution of predator nymphs per seedling

The evenness of egg distribution per seedling plant was not influenced by species or release rate but was influenced by food provisioning: when predatory mirid bugs were provided with food, females not only laid more eggs (Fig. 1) but also distributed them more evenly among plants (Fig. 2), a positive characteristic of the pre-planting method because biological control by mirid bugs in the greenhouse will be more homogeneous.

Injury to tomato seedlings

During all the experiments no injuries caused by the two *Dicyphus* species were observed on the seedling plants. Any significant effect of the factors studied or their double interactions on plant development (length of leaves, number of leaflets per leaf, and total number of leaves per plant) was found. In Supplementary Table S1, detailed statistical analysis results are shown.

Fig. 2 Coefficient (+ SE) of variation (SD/mean of the number of nymphs per tomato seedling) when 0.5 and 1 females of *D. errans* and *D. bolivari* were initially released in tents with six tomato seedling plants and fed with *E. kuehniella* eggs (Diet⁺) or not (Diet⁻) at 25 °C, 16:8 h L:D, and 55–70% of RH. Females were allowed to lay eggs on tomato seedlings for five days. Asterisks (*) indicate significant differences ($P < 0.05$) and n.s indicates not significant differences ($P > 0.05$)



Discussion

Dicyphus bolivari and *D. errans* were able to successfully develop under winter and summer conditions, indicating that both *Dicyphus* species may be used throughout the year, considering their development and survival. This result was also reported in *D. hesperus* (Knight), which established well in both autumn–winter and summer conditions on tomatoes (Calvo et al. 2016). Nymph development time under laboratory standard (summer) conditions (25 °C, 16:8 h L:D; feeding on *E. kuehniella* eggs) for the case of *D. bolivari* (18.9 days) was similar to that reported in the literature for the species here studied under similar conditions and diet. For *D. maroccanus*, Abbas et al. (2014) recorded a similar development length (19.5 days). However, in the case of *D. errans* (16.3 days), the development time we observed was almost four days shorter than in the study of the same species by Ingegno et al. (2013), who reported 20.4 days. These results were obtained under similar conditions like ours. In comparison with the current commercially used predatory bugs, *M. pygmaeus* and *N. tenuis*, nymph development times observed for *D. bolivari* and *D. errans* were approximately six and three days longer, respectively, than for *N. tenuis* and three and 0.5 days than for *M. pygmaeus* (Mollá et al. 2014) at the same temperature and RH conditions. In

winter conditions, no data are available from the literature for *N. tenuis* and *M. pygmaeus*. Therefore, from the point of view of a quicker predator establishment, the two *Dicyphus* spp. here studied do not seem to improve *N. tenuis* and *M. pygmaeus* characteristics at summer conditions. However, it should be considered that a longer nymphal development time may increase biological control, since predators can remain on the crop longer and consequently consume more prey (Abbas et al. 2014). Our results show that diet influences nymph development time of both *Dicyphus* species under both climate conditions, with nymph development being shorter when a mixed diet (tomato leaf with *B. tabaci* nymphs + *E. kuehniella* eggs) was provided. Faster nymph development of *Dicyphus* may be due to synergistic effects of the two diets, providing different nutritional value and composition for the development of nymphs (Coll and Guershon 2002). Our results are similar to those of other studies that observed that mixed diets of prey and plant food enhance pest control by generalist predators (Messelink et al. 2008) and different life history traits of polyphagous predators, including decreasing development time in coccinellids (Lundgren 2009) and in *Chrysoperla carnea* Stephens (Patt et al. 2003). Furthermore, mixed diets may affect other parameters not covered in the present study, such as shorter pre-oviposition periods and increased female fecundity, as observed in species belonging to other insect taxa such as Hemiptera and Coleoptera (Berkvens et al. 2008; Kajita and Evans 2010; Moya-Raygoza and Garcia-Medina 2010). Higher predator performance in mixed rather than single diets could also explain the advantages of omnivory, as suggested by Marques et al. (2015), a common behaviour in *Dicyphus* spp. and other predatory mirid bugs.

When adults of *D. bolivari* and *D. errans* were released on pure tomato seedlings which were neither occupied with other entomophagous nor phytophagous arthropods, they were able to lay eggs and generate offspring, as observed in studies carried out with the two commercially used predatory mirids, *N. tenuis* and *M. pygmaeus* (Calvo et al. 2012b; Lenfant et al. 2000; Perdakis et al. 2015). However, *N. tenuis* caused injuries to the leaves of tomato plants (necrotic rings) soon after transplantation at release rates above 0.5 predators per plant, even when *B. tabaci* was available as prey in a quantity similar to that provided

in this work (Calvo et al. 2012b). In our study, neither of the two *Dicyphus* species caused injuries to the seedlings, even when a high density of individuals was released (two individuals per plant) and in the absence of food. Even two weeks after predator removal, no negative effect on plant development (number and length of leaves) was observed compared with plants without predators. That no damage was caused by mirid bugs on tomato seedlings can be considered as a very positive point, since seedlings are very tender and injuries can weaken the plant, affecting the future plant development and causing economic losses (Silva et al. 2017). The release of both predatory mirid bug species with numbers of 0.5 and 1 females per tomato seedling for five days led to an offspring of 1st instar nymphs that we assume to be sufficient to ensure an early establishment of predatory mirid bugs on greenhouse tomatoes and effective pest suppression. Lower rates of the mirid bug can be used in function of expected abundance of phytophagous arthropods and crop season. For instance, in late summer or early autumn when pest arthropods are abundant, higher rates of mirid bugs may be required in order to carry out an effective biological control, whereas in spring, when the abundance of pest arthropods is lower, a higher number of predatory mirid bugs released on tomato seedlings can be negative because of a shortage of prey which may cause the predators to abandon the greenhouse or to feed on the crop plants.

Provision of food proved to be a key point in this method, since by numerical response it may increase the number of produced nymphs per plant almost four times. In fact, this result was expected since some studies have shown that the application of *E. kuehniella* eggs enhances survival and reproductive parameters of coccinellids (De Clercq et al. 2006) and predatory mirid bugs (Mollá et al. 2014). Moreover, the dispersed provision of *E. kuehniella* eggs spread over many plants throughout the nursery improved the distribution of nymphs among plants in addition to the increased number of predatory mirid bugs established in the greenhouse, i.e., a better control can be expected.

The results of this study with the two *Dicyphus* species complement those of previous studies on the ability of both species to suppress tobacco whitefly populations and *T. absoluta* in laboratory and semi-field trials (Gabarra et al. 1988; Albajes et al. 1996; Tavella et al. 1997; Ingegno et al. 2017; Castañé et al.

2018) and may suggest that these two predatory mirids may be an alternative to *M. pygmaeus* and *N. tenuis* under some circumstances. To validate these results in real conditions, currently we are carrying out some field trials with the two *Dicyphus* spp. inoculated in the nursery in comparison with the use of this technique in *N. tenuis* and *M. pygmaeus*.

In conclusion, the development and survival of *D. bolivari* and *D. errans* are suitable for them to be used both under Mediterranean different conditions. Pre-planting inoculation of these predatory mirid bugs in the nursery has proved to be feasible, with no risk of damage to seedling plants as occurs with other predatory mirids on different plants. This method may be particularly recommended when an earlier establishment of the predator in the greenhouse is necessary due to rapid development of pest populations. Moreover, the use of this method makes the release of additional predatory mirid bugs unnecessary or may at least save a number of releases. Provision of food—e.g. *Ephestia* eggs like in this work—in the nursery improves the mirid bug offspring and its distribution per plant. The rate of one couple of predatory bugs per tomato seedling results in a sufficient number of offspring (nymphs) on seedlings for an early establishment of the mirid bugs in the greenhouse. At least at the release rates and tomato variety used in the experiment, the risk of injuries on seedling plants by either of the two predators tested is negligible.

Acknowledgements This work was funded by the EU Horizon 2020 projects EMPHASIS (Grant Agreement No 634179) and EUCLID (Grant Agreement No 633999). E.E was funded by the Spanish Government project AGL2014-53970-C2-1-R.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Abbas S, Perez-Hedo M, Colazza S, Urbaneja A (2014) The predatory mirid *Dicyphus maroccanus* as a new potential biological control agent in tomato crops. *BioControl* 59:565–574
- Albajes R, Alomar O (1999) Current and potential uses of polyphagous predators. In: Albajes R, Gullino ML, van Lenteren JC, Elad Y (eds) Integrated pest and disease management in greenhouse crops. Kluwer Academic Publishers, Dordrecht, pp 265–273
- Albajes R, Alomar O (2004) Facultative predators. In: Capinera JL (ed) Encyclopedia of entomology, vol 2. Kluwer Academic Publishers, Dordrecht, pp 818–822
- Albajes R, Alomar O, Riudavets J, Castañé C, Arnó J, Gabarra R (1996) The mirid bug *Dicyphus tamaninii*: an effective predator for vegetables crops. *IOBC/WPRS Bull* 19:1–4
- Alomar O, Albajes R (1996) Greenhouse whitefly (Homoptera: Aleyrodidae) predation and tomato fruit injury by the zoophytophagous predator *Dicyphus tamaninii* (Heteroptera: Miridae). In: Alomar O, Wiedenmann RN (eds) Zoophytophagous heteroptera: implications for life history and integrated pest management. Thomas Say Publications in Entomology, Lanham, pp 155–177
- Barnadas I, Gabarra R, Albajes R (1998) Predatory capacity of two mirid bugs preying on *Bemisia tabaci*. *Entomol Exp Appl* 86:215–219
- Berkvens N, Bonte J, Berkvens D, Deforce K, Tirry L, De Clercq P (2008) Pollen as an alternative food for *Harmonia axyridis*. *BioControl* 53:201–210
- Bueno VHP, van Lenteren JC (2010) Biological control of pests in protected cultivation: implementation in Latin America and successes in Europe. *Memorias, XXXVII Congreso Sociedad Colombiana de Entomología*, Bogota, D.C., 30 June–2 July 2010:261–269
- Calvo FJ, Lorente MJ, Stansly PA, Belda JE (2012a) Preplant release of *Nesidiocoris tenuis* and supplementary tactics for control of *Tuta absoluta* and *Bemisia tabaci* in greenhouse tomato. *Entomol Exp Appl* 143:111–119
- Calvo FJ, Bolckmans K, Belda JE (2012b) Release rate for a pre-plant application of *Nesidiocoris tenuis* for *Bemisia tabaci* control in tomato. *BioControl* 57:809–817
- Calvo FJ, Torres-Ruiz A, Velázquez-González JC, Rodríguez-Leyva E, Lomeli-Flores JR (2016) Evaluation of *Dicyphus hesperus* for biological control of sweet potato whitefly and potato psyllid on greenhouse tomato. *BioControl* 61:415–424
- Casadevall M, Bordas E, Albajes R (1979) La mosca blanca de los invernaderos, *Trialeurodes vaporariorum*, en El Mar-esme. I, Resultados preliminares de lucha integrada en un cultivo de tomate. *INIA Ser Prot Veg* 11:45–56
- Castañé C, Arnó J, Gabarra R, Alomar O (2011) Plant damage to vegetable crops by zoophytophagous mirid predators. *Biol Control* 59:22–29
- Castañé C, Arnó J, Riudavets J, Albajes J, Gabarra R (2018) Efficacy of two predatory mirid bugs as candidates for the control of tomato pests. *IOBC/WPRS Bull* (in press)
- Coll M, Guershon M (2002) Omnivory in terrestrial arthropods: mixing plant and prey diets. *Annu Rev Entomol* 47:267–297
- De Clercq P, Bonte M, van Speybroeck K, Bolckmans K, Deforce K (2006) Development and reproduction of *Adalia bipunctulata* (Coleoptera: Coccinellidae) on eggs of *Ephestia kuehniella* (Lepidoptera: Phycitidae) and pollen. *Pest Manag Sci* 61:1129–1132

- Gabarra R, Castañé C, Bordas E, Albajes R (1988) *Dicyphus tamaninii* Wagner as a beneficial insect and pest of tomato crops in Catalonia. Entomophaga 33:219–228
- Ingegno BL, Ferracini C, Gallinotti D, Alma A, Tavella L (2013) Evaluation of the effectiveness of *Dicyphus errans* (Wolff) as predator of *Tuta absoluta* (Meyrick). Biol Control 67:246–252
- Ingegno BL, Bodino N, Leman A, Messelink GJ, Tavella L (2017) Predatory efficacy of *Dicyphus errans* on different prey. Acta Hort 1164:425–430
- Kajita Y, Evans EW (2010) Relationship of body size, fecundity, and invasion success among predatory lady beetles (Coleoptera: Coccinellidae) inhabiting alfalfa fields. Ann Entomol Soc Am 103:750–756
- Lenfant C, Ridray G, Schoen L (2000) Biopropagation of *Macrolophus caliginosus* (Wagner) for a quicker establishment in southern tomato greenhouses. IOBC/WPRS Bull 23:247–252
- Lundgren JG (2009) Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. Biol Control 51:294–305
- Marques RV, Sarmiento RA, Lemos F, Pedro-Neto M, Sabelis MW, Venzon M, Pallini A, Janssen A (2015) Active prey mixing as an explanation for polyphagy in predatory arthropods: synergistic dietary effects on egg production despite a behavioural cost. Funct Ecol 29:1317–1324
- Messelink GJ, van Maanen R, van Steenpaal SEF, Janssen A (2008) Biological control of thrips and whiteflies by a shared predator: two pests are better than one. Biol Control 44:372–379
- Messelink GJ, Bennison J, Alomar O, Ingegno BL, Tavella L, Shipp L, Palevsky E, Wäckers FL (2014) Approaches to conserving natural enemy populations in greenhouse crops: current methods and future prospects. BioControl 59:377–393
- Mollá O, Biondi A, Alonso-Valiente M, Urbaneja A (2014) A comparative life history study of two mirid bugs preying on *Tuta absoluta* and *Ephestia kuehniella* eggs on tomato crops: implications for biological control. BioControl 59:175–183
- Moya-Raygoza G, Garcia-Medina C (2010) Comparison of fecundity and body size of Mexican and Argentinian populations of *Dalbulus maidis* (Hemiptera: Cicadellidae). Ann Entomol Soc Am 103:544–547
- Onillon JC (1990) The use of natural enemies for the biological control of whiteflies. In: Gerling D (ed) Whiteflies: their bionomics, pest status and management. Intercept Ltd, Andover, pp 287–314
- Patt JM, Wainwright SC, Hamilton GC, Whittinghill D, Bosley K, Dietrick J, Lashomb JH (2003) Assimilation of carbon and nitrogen from pollen and nectar by a predaceous larva and its effects on growth and development. Ecol Entomol 28:717–728
- Perdikis DC, Arvaniti KA, Paraskevopoulos A, Grigoriou A (2015) Pre-plant release enhanced the earlier establishment of *Nesidiocoris tenuis* in open field tomato. Entomol Helv 24:11–21
- R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Version 2.15.0. Vienna, Austria
- Riudavets J, Castañé C (1994) Abundance and host plant preferences for oviposit (Heteroptera: Anthocoridae) along the Mediterranean coast of Spain. IOBC/WPRS Bull 17:230–236
- Sanchez JA, Cassis G (2018) Towards solving the taxonomic impasse of the biocontrol plant bug subgenus *Dicyphus* (*Dicyphus*) (Insecta: Heteroptera: Miridae) using molecular, morphometric and morphological partitions. Zool J Linn Soc 184:330–406
- Silva DB, Bueno VHP, Calvo FJ, van Lenteren JC (2017) Do nymphs and adults of three Neotropical zoophytophagous mirids damage leaves and fruits of tomato? Bull Entomol Res 107:200–207
- Tavella L, Alma A, Sargiotto C (1997) Samplings of Miridae Dicyphinae in tomato crops of Northwestern Italy. IOBC/WPRS Bull 20:249–256
- Trottin-Caudal Y, Baffert V, Leyre JM, Hulas N (2012) Experimental studies on *Tuta absoluta* (Meyrick) in protected tomato crops in France: biological control and integrated crop protection. EPPO Bull 42:234–240
- Urbaneja A, González-Cabrera J, Arnó J, Gabarra R (2012) Prospects for the biological control of *Tuta absoluta* in tomatoes of the Mediterranean basin. Pest Manag Sci 68:1215–1222
- van Lenteren JC (2012) The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. BioControl 57:71–84
- van Lenteren JC, Woets J (1988) Biological and integrated pest control in greenhouses. Annu Rev Entomol 33:239–269
- van Lenteren JC, Alomar O, Ravensberg WJ, Urbaneja A (2019) Biological control agents for control of pest in greenhouses. In: Gullino ML, Albajes R, Nicot P (eds) Integrated pest and disease management in greenhouse crops. Springer Verlag GmbH, Heidelberg
- Venables WN, Ripley BD (2002) Modern applied statistics with S. Springer, New York

Filipe Madeira is a post-doc researcher at the University of Lleida—Agrotecnio Centre. His research is devoted to biological control of insect pests and relationships between insects and landscape in agroecosystems.

Eva Edo is a post-graduate researcher at the Laboratory of Entomology, University of Lleida and she is working on ecology of insects and weeds.

Samuel Sossai is a post-graduate researcher at the Laboratory of Entomology, University of Lleida and he is working on biological control of insect pests and relationships between insects and landscape in agroecosystems.

Joan Callizo was a master student at the Laboratory of Entomology, University of Lleida and he was working on ecology of insects. Part of the results are from his master thesis.

Ramon Albajes is professor in entomology at the University of Lleida. He has been working on biological control in greenhouse crops for many years and has published several articles on the topic.